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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/586,896	10/16/2006	Ferdinand Hermann Bahlmann	P/2107-297	4965

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OSTROLENK FABER GERB & SOFFEN
1180 AVENUE OF THE AMERICAS
NEW YORK, NY 100368403

EXAMINER	
DEBERRY, REGINA M	

ART UNIT	PAPER NUMBER
1647	

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09/30/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/586,896	BAHLMANN ET AL.	
	Examiner	Art Unit	
	Regina M. DeBerry	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 August 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *4-6,10,15,19,32,39,40,45,49,52-54 and 57-64* is/are pending in the application.

4a) Of the above claim(s) *4-6,10,15,19,32,39,40,45,49,52,53 and 58-64* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *54 and 57* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 04 August 2010 has been entered.

Status of Application, Amendments and/or Claims

The amendment and Applicant's arguments, filed 04 August 2010, have been entered in full. Claims 4-6, 10, 15, 19, 32, 39, 40, 45, 49, 52, 53, 58-64 are withdrawn from consideration as being drawn to a non-elected invention. Claims 1-3, 7-9, 11-14, 16-18, 20-31, 33-38, 41-44, 46-48, 50, 51, 55, 56 are canceled. Claim 54 is amended. Claims 54 and 57 are under examination.

Withdrawn Objections And/Or Rejections

The rejection to claims 54 and 57 under 35 U.S.C. 112, first paragraph, written description requirement, new matter, as set forth at pages 9-10 of the previous Office Action (28 April 2010), is *withdrawn* in view of the amendment (04 August 2010).

Claim Rejections-35 USC § 112, First Paragraph, Scope of Enablement

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 54 and 57 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

"a method for treating acute or chronic renal failure in a human or animal patient exhibiting a) at least one dysfunction of endothelial cells, b) hypertension and c) at least one end-organ damage, wherein the at least one end-organ damage is selected from the group consisting of left ventricular hypertrophy, microalbuminuria, proteinuria and glomerular filtration rate of 30 to 80 ml/min, said method comprising administering to said patient a pharmaceutical composition comprising a subpolycythemic dosage of **erythropoietin or Aranesp**, wherein the acute or chronic renal failure is thereby treated in said human or animal patient **by at least one of diminution or slowing of the damage to kidney tissue**

does not reasonably provide enablement for:

"a method for treating acute or chronic renal failure in a human or animal patient exhibiting a) at least one dysfunction of endothelial cells, b) hypertension and c) at least one end-organ damage, wherein the at least one end-organ damage is selected from the group consisting of left ventricular hypertrophy, microalbuminuria, proteinuria and glomerular filtration rate of 30 to 80 ml/min, said method comprising administering to said patient a pharmaceutical composition comprising a subpolycythemic dosage of **at least one of erythropoietin and a derivative thereof**, wherein the acute or chronic renal failure is thereby treated in said human or animal patient **by at least one of**

prevention of the damage to kidney tissue and regeneration of damage kidney tissue.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims. The basis for this rejection is set forth at pages 3-8 of the previous Office Action (28 April 2010).

Applicant argues that the method is directed to the proposition that a particular group of patients, as recited in claim 54 for example, can be treated in a particular manner, namely using a subpolycythemic dosage of EPO which does not raise the hematocrit value and which leads to the prevention, diminution or slowing of damage to kidney tissue and/or to regeneration of damaged kidney tissue. Applicant maintains that the effects attributable to the subject method rely on the use of EPO which according to the specification (pages 21-25), constitutes a protein with the biological activity of EPO. Applicant maintains that the protein is not decisive in achieving the aims of Applicants' process-so long as a protein is used that has the biological activity of EPO. Applicant argues that the biological activity of EPO is well known among those having ordinary skill in the art, which enables the skilled artisan to readily determine whether a specific protein falls within the scope of “at least one of erythropoietin or a derivative thereof”. Applicant argues that the description set forth at pages 21-25 provide specific examples of some EPO derivatives useful in the present invention.

Applicant’s arguments have been fully considered but are not found persuasive. As was stated in the previous Office Action, page 23 of the instant specification states,

“the differences between an erythropoietin derivative and native erythropoietin may arise, for example, through mutations such as deletions, substitutions, insertions, additions, base exchanges and/or recombinations of the nucleotide sequences coding the erythropoietin amino acid sequences" (page 23). It is well recognized in the art that any modification (even a "conservative" substitution) to a critical structural region of a protein is likely to alter its functional properties. In order to make a sequence variant with the reasonable assurance that it would have the desirable properties of the invention, the artisan would need to know which regions of the disclosed polypeptide are responsible for the interactions underlying its biological function(s). For sequences having one or two substitutions, for example, the artisan would reasonably expect that many of the possible variants would retain functional properties comparable to those of the unmodified protein, and it would require only routine manipulations to make and test a reasonably representative sampling of the possible variants. However, as the number of modified sites increases, the number of possible variants, and hence the degree of experimentation required, increases exponentially. Additionally, as plural substitutions are introduced, their interactions with each other and their effects on the structure and function of the protein become progressively less predictable. Reasonable correlation must exist between the scope of the claims and scope of enablement set forth. Contrary to Applicant’s assertion, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope.

Lastly, instant claim 54 has been amended to recite, "..wherein the acute or chronic renal failure is thereby treated in said human or animal patient by at least one of

prevention, diminution or slowing of the damage to kidney tissue and *regeneration* of damage kidney tissue". The instant specification is not enabling for the full scope of the claims. The Examples teach the effects of administered EPO in subjects with renal anemia and chronic renal failure. Example 1 teaches increased mobilization and an increase in the number of endothelial progenitors cells in patients with renal anemia (as a consequence of renal disease in pre-terminal renal failure stage). Patients are treated with 5000 IU of rhEPO. Examples 3 and 4 teach the reduction in the progression of chronic and acute renal failure upon administering EPO in rat animal models (pages 82-84). The specification fails to teach that kidney damage has been prevented. Prevent means to completely stop a condition from occurring. "Prevention" is not a relative term, it is total. A very high degree of evidence is required, which is accepted in the art, that an absolute protection from the pathology exists over an extended period of time. Furthermore, the specification fails to demonstrate any type of regeneration of damaged kidney tissue. The scientific reasoning and evidence as a whole indicates that the rejection should be maintained.

Claim Rejections-35 USC § 103(a)

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 54 and 57 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Jungers et al., Nephrology Dialysis Transplantation 16:307-312 (2001) in view of Stehouwer et al., Nederlands tijdschrift voor geneeskunde. Abstract in English. Vol. 141/No. 34:1649-53 (Aug 23 1997). The basis for this rejection is set forth at pages 10-13 of the previous Office Action (28 April 2010).

Applicant discusses the Jungers reference. Applicant concludes by stating that the effects in the Jungers et al. reference are clearly due to the hemoglobin-increasing effect of the moderate EPO doses. Applicant argues that the presently claimed method is not intended for increasing the subject's hemoglobin (Hb) value. Applicant argues that the claims specifically recite the usage of a subpolycythemic dosage of EPO, which according to the teachings provided in the present application, refers to a dosage which does not influence the Hb value. Applicant states that claim 54, prior to amendment herein, referred explicitly to an EPO dosage of 1-90 IU/kg body weight per week, but this numerical range only spans the principally applicable dosage range, which is further

limited by the specific requirement that the dosage to be applied is a subpolycythemic dosage. Applicant maintains that a subpolycythemic dosage is not limited to the indicated range. Applicant states that the decisive feature is that the dosage be subpolycythemic as taught on pages 44-45 of the specification. Applicant states that they have amended claim 54 to remove the preferred dosage range, while retaining the language that the dosage must be subpolycythemic, i.e., one that does not lead to an increase in the hematocrit of a subject. Applicant asserts that since Jungers et al. clearly link the beneficial effect of prolonging the pre-dialysis phase to the correction of an anemic condition, namely by raising the Hb level, it would be totally unexpected by one having ordinary skill in the art that a dosage which does not raise or alter the Hb value, i.e. subpolycythemic dose, would have any effect on the conditions specified in the claims. Applicant argues that the claimed method is not directed to the treatment of anemia but instead teaches one of ordinary skill in the art that the claimed subpolycythemic dose of EPO, which does not cause the Hb level to rise, has structural effects on the damaged kidney tissue and prevents or reduces damage to the kidney itself. Applicant argues that the indicated effect has nothing to do with the treatment of anemia because its exerted on the level of the renal tissue, namely at the cellular level. The effect is, therefore, totally unexpected based on the teachings contained in Jungers et al. Applicant argues that the Stehouwer et al. reference does not remedy the deficiencies of the Jungers et al. reference.

Applicant's arguments have been fully considered but are not found persuasive. The specification defines subpolycythemic doses as doses that do not lead to an

increase of ***hematocrit*** *not hemoglobin.* Hematocrit is the proportion of blood volume that is occupied by red blood cells (see below). Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells (see below). The specification states, "**all of the foregoing doses provided according to the invention**, for example of 1-2000 units (IU)/week per patient, especially, for example, of 550-2000 IU/week per patient, **are subpolycythemic doses, or in other words doses that do not lead to an increase of the hematocrit**.."(bottom of page 44). "The subpolycythemic doses provide according to the invention correspond to weekly doses of about 1-90 IU of EPO/kg of body weight (top of page 45). **"All of the foregoing doses provided according to the invention**, for example of 0.01-90 units (IU)/kg/week per patient, especially, for example, of 0.01-50 IU/kg/week per patient, **are subpolycythemic doses, or in other words doses that do not lead to an increase of the hematocrit.."**(bottom of page 46-top of page 47). Jungers et al. teach that a slowing of progression of CRF and a decrease of blood pressure was observed in EPO treated patients. Jungers et al. teach that 20 chronic renal failure (CRF) patients received 54.3+_ 16.5 U/kg/week of EPO. Thus, by definition of the instant specification, Jungers et al. administers a subpolycythemic dose. The Stehouwer reference was used to teach that microalbuminuria, hypertension, endothelial dysfunction and an increased risk of left ventricular hypertrophy are aspects in CRF patients. The scientific reasoning and evidence as a whole indicates that the rejection should be maintained.

Hematocrit

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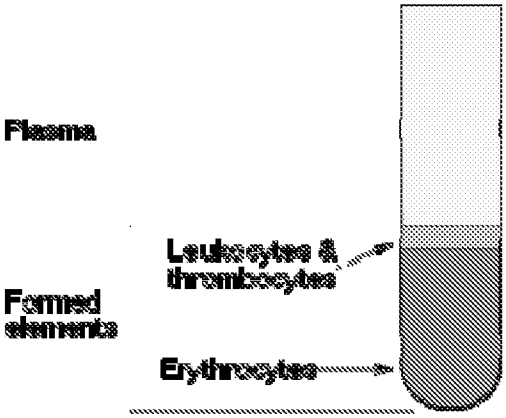
Jump to: navigation, search

This article **needs additional citations for verification.**
Please help improve this article by adding reliable references. Unsourced material may be challenged and removed. *(March 2008)*



The **hematocrit** (**Ht** or **HCT**) or **packed cell volume** (**PCV**) or **erythrocyte volume fraction** (EVF) is the proportion of blood volume that is occupied by red blood cells. It is normally about 48% for men and 38% for women.[1] It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count.

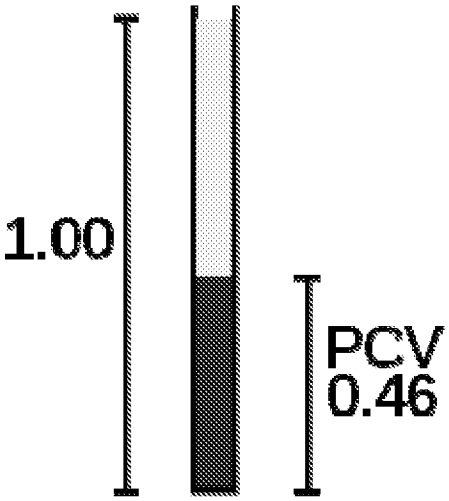
In mammals, hematocrit is independent of body size.

The term "hematocrit" (British English: haematocrit) was coined in 1903. Its roots stem from the Greek words hema (Gr αἷμα)—blood, and krites (Gr κριτής), judge—meaning to gauge or judge the blood.

Contents

[hide]

[edit] Measurement methods



Packed cell volume diagram

The packed cell volume (PCV) can be determined by centrifuging heparinized blood in a capillary tube (also known as a microhematocrit tube) at 10,000 RPM for five minutes.[2] This separates the blood into layers. The volume of packed red blood cells divided by the total volume of the blood sample gives the PCV. Because a tube is used, this can be calculated by measuring the lengths of the layers.

With modern lab equipment, the hematocrit is calculated by an automated analyzer and not directly measured. It is determined by multiplying the red cell count by the mean cell volume. The hematocrit is slightly more accurate as the PCV includes small amounts of blood plasma trapped between the red cells. An estimated hematocrit as a percentage may be derived by tripling the hemoglobin concentration in g/dL and dropping the units.[3] The hemoglobin level is the measure used by blood banks.[*clarification needed*][*citation needed*]

There have been cases in which the blood for testing was inadvertently drawn proximal to an intravenous line that was infusing packed red cells or fluids. In these situations, the hemoglobin

level in the blood sample will not be the true level for the patient because the sample would contain a large amount of the infused material rather than what is diluted into the circulating whole blood. That is, if packed red cells are being supplied, the sample will contain a large amount of those cells and the hematocrit will be artificially very high. On the converse, if saline or other fluids are being supplied, the blood sample would be diluted and the hematocrit will be artificially low.

[edit] Elevated hematocrit

In cases of dengue fever, a high hematocrit is a danger sign of an increased risk of dengue shock syndrome.

Polycythemia vera (PV), a myeloproliferative disorder in which the bone marrow produces excessive numbers of red cells, is associated with elevated hematocrit.

Chronic obstructive pulmonary disease (COPD) and other pulmonary conditions associated with hypoxia may elicit an increased production of red blood cells. This increase is mediated by the increased levels of erythropoietin by the kidneys in response to hypoxia.

Professional athletes' hematocrit levels are measured as part of tests for blood doping or Erythropoietin (EPO) use; the level of hematocrit in a blood sample is compared with the long-term level for that athlete (to allow for individual variations in hematocrit level), and against an absolute permitted maximum (which is based on maximum expected levels within the population, and the hematocrit level that causes increased risk of blood clots resulting in strokes or heart attacks).

Anabolic Androgenic Steroid (AAS) use can also increase the amount of RBCs and, therefore, impact the hematocrit, in particular the compounds boldenone and oxymethelone.

If a patient is dehydrated, the hematocrit may be elevated.

[edit] Lowered hematocrit

Lowered hematocrit can imply significant hemorrhage.

The mean corpuscular volume (MCV) and the red cell distribution width (RDW) can be quite helpful in evaluating a lower-than-normal hematocrit, because it can help the clinician determine whether blood loss is chronic or acute. The MCV is the size of the red cells and the RDW is a relative measure of the variation in size of the red cell population. A low hematocrit with a low MCV with a high RDW suggests a chronic iron-deficient erythropoiesis, but a normal RDW suggests a blood loss that is more acute, such as a hemorrhage.

Groups of individuals at risk for developing anemia include:

- infants without adequate iron intake

- children going through a rapid growth spurt, during which the iron available cannot keep up with the demands for a growing red cell mass
- women in childbearing years with an excessive need for iron because of blood loss during menstruation
- pregnant women, in whom the growing fetus creates a high demand for iron
- patients with chronic kidney disease, as their kidneys no longer secrete sufficient levels of the hormone erythropoietin, which stimulates red blood cell production by the bone marrow.

Hemoglobin

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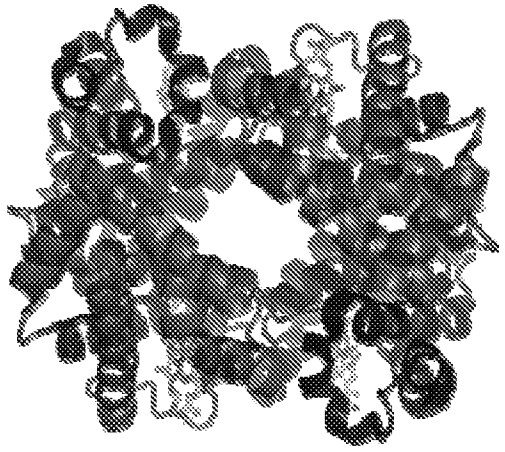
Hemoglobin, human, adult

(heterotetramer, $(\alpha\beta)_2$)



Structure of human hemoglobin. The protein's α and β subunits are in red and blue, and the iron-containing heme groups in green. From PDB 1GZX

Proteopedia Hemoglobin

Protein type		metalloprotein, globulin
Function		oxygen-transport
Cofactor(s)		heme (4)
Subunit Name	Gene	Chromosomal Locus
Hb-α1	HBA1	Chr. 16 p13.3
Hb-α2	HBA2	Chr. 16 p13.3
Hb-β	HBB	Chr. 11 p15.5

Hemoglobin (also spelled **haemoglobin** and abbreviated **Hb** or **Hgb**) is the iron-containing oxygen-transport metalloprotein in the red blood cells of vertebrates[1] and the tissues of some invertebrates. Hemoglobin in the blood is what transports oxygen from the lungs or gills to the rest of the body (i.e. the tissues) where it releases the oxygen for cell use.

In mammals the protein makes up about 97% of the red blood cell's dry content, and around 35% of the total content (including water)[*citation needed*]. Hemoglobin has an oxygen binding capacity between 1.36 and 1.37 ml O_2 per gram of hemoglobin,[2] which increases the total blood oxygen capacity seventyfold.[3]

Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.[4]

Contents

[hide]

[edit] Research history

The oxygen-carrying protein hemoglobin was discovered by Hünefeld in 1840.[5] In 1851,[6] Otto Funke published a series of articles in which he described growing hemoglobin crystals by

successively diluting red blood cells with a solvent such as pure water, alcohol or ether, followed by slow evaporation of the solvent from the resulting protein solution.[7] Hemoglobin's reversible oxygenation was described a few years later by Felix Hoppe-Seyler.[8]

In 1959 Max Perutz determined the molecular structure of hemoglobin by X-ray crystallography.[9][10] This work resulted in his sharing with John Kendrew the 1962 Nobel Prize in Chemistry.

The role of hemoglobin in the blood was elucidated by physiologist Claude Bernard. The name *hemoglobin* is derived from the words *heme* and *globin*, reflecting the fact that each subunit of hemoglobin is a globular protein with an embedded heme (or haem) group. Each heme group contains one iron atom, that can bind one oxygen molecule through ion-induced dipole forces. The most common type of hemoglobin in mammals contains four such subunits.

[edit] Genetics

Hemoglobin consists mostly of protein (the "globin" chains), and these proteins, in turn, are composed of sequences of amino acids. These sequences are linear, in the manner of letters in a written sentence or beads on a string. In all proteins, it is the variation in the type of amino acids in the protein sequence of amino acids, which determine the protein's chemical properties and function. This is true of hemoglobin, where the sequence of amino acids may affect crucial functions such as the protein's affinity for oxygen.

There is more than one hemoglobin gene. The amino acid sequences of the globin proteins in hemoglobins usually differ between species, although the differences grow with the evolutionary distance between species. For example, the most common hemoglobin sequences in humans and chimpanzees are nearly identical, differing by only one amino acid in both the alpha and the beta globin protein chains. These differences grow larger between less closely related species.

Even within a species, different variants of hemoglobin always exist, although one sequence is usually a "most common" one in each species. Mutations in the genes for the hemoglobin protein in a species result in hemoglobin variants.[11][12] Many of these mutant forms of hemoglobin cause no disease. Some of these mutant forms of hemoglobin, however, cause a group of hereditary diseases termed the *hemoglobinopathies*. The best known hemoglobinopathy is sickle-cell disease, which was the first human disease whose mechanism was understood at the molecular level. A (mostly) separate set of diseases called thalassemias involves underproduction of normal and sometimes abnormal hemoglobins, through problems and mutations in globin gene regulation. All these diseases produce anemia.[13]

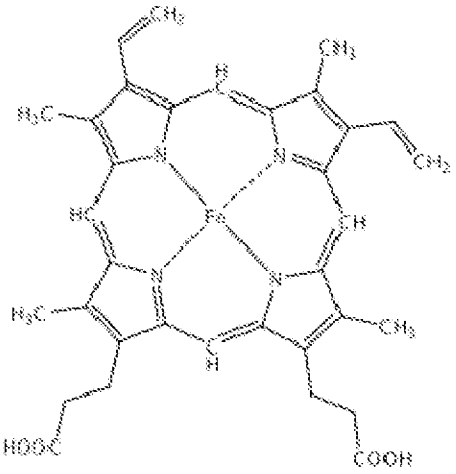
Variations in hemoglobin amino acid sequences, as with other proteins, may be adaptive. For example, recent studies have suggested genetic variants in deer mice that help explain how deer mice that live in the mountains are able to survive in the thin air that accompanies high altitudes. A researcher from the University of Nebraska-Lincoln found mutations in four different genes that can account for differences between deer mice that live in lowland prairies versus the mountains. After examining wild mice captured from both highlands and lowlands, it was found that: the genes of the two breeds are “virtually identical–except for those that govern the oxygen-

carrying capacity of their hemoglobin". "The genetic difference enables highland mice to make more efficient use of their oxygen", since less is available at higher altitudes, such as those in the mountains.[14] Mammoth hemoglobin featured mutations that allowed for oxygen delivery at lower temperatures, thus enabling mammoths to migrate to higher latitudes during the Pleistocene.[15]

[edit] Synthesis

Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol.[16] Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name).

[edit] Structure



Heme group

Hemoglobin exhibits characteristics of both the tertiary and quaternary structures of proteins.[17] Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape.[18] Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.[17]

In most humans, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement, so called because this arrangement is the same folding motif used in other

heme/globin proteins such as myoglobin.[19][20] This folding pattern contains a pocket that strongly binds the heme group.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together with the iron ion bound in the center.[21] The iron ion, which is the site of oxygen binding, coordinates with the four nitrogens in the center of the ring, which all lie in one plane. The iron is bound strongly to the globular protein via the imidazole ring of the F8 histidine residue below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond,[22] completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron.

Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions, but is actually bound to the protein chains of the structure.

The iron ion may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (methemoglobin) (Fe^{3+}) cannot bind oxygen.[23] In binding, oxygen temporarily and reversibly oxidizes (Fe^{2+}) to (Fe^{3+}) while oxygen temporally turns into superoxide, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe^{3+} is protonated the hemoglobin iron will remain oxidized and incapable to bind oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin type is a tetramer (which contains 4 subunit proteins) called **hemoglobin A**, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha_2\beta_2$. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of about 17,000 daltons, for a total molecular weight of the tetramer of about 68,000 daltons (64,458 g/mol)[24]. Thus, 1 g/dL = 0.01551 mmol/L. Hemoglobin A is the most intensively studied of the hemoglobin molecules.

In human infants, the hemoglobin molecule is made up of 2 α chains and 2 gamma chains. The gamma chains are gradually replaced by β chains as the infant grows.[25]

The four polypeptide chains are bound to each other by salt bridges, hydrogen bonds, and hydrophobic interactions. There are two kinds of contacts between the α and β chains: $\alpha_1\beta_1$ and $\alpha_1\beta_2$.

In general, hemoglobin can be saturated with oxygen molecules (oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin).[26] *Oxyhemoglobin* is formed during physiological respiration when oxygen binds to the heme component of the protein hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized in aerobic glycolysis and in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH.[27]

Deoxyhemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This accounts for hemoglobin's red color and deoxyhemoglobin's blue color. This difference is used for measurement of the amount of oxygen in patient's blood by an instrument called pulse oximeter.

NEW CLAIM REJECTIONS/OBJECTIONS

Claim Rejections - 35 USC § 112, Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 54 and 57 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 54 recites the limitation, “prevention”. It is unclear how to prevent a condition that the patient is required to have by the claim for treatment. That is to say how are acute or chronic renal failure treated in a human or animal patient if damage to the kidney tissue is being prevented. The metes and bounds of the instant claims cannot be determined.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Regina M. DeBerry whose telephone number is (571) 272-0882. The examiner can normally be reached on 9:00 a.m.-6:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey J. Stucker can be reached on (571) 272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Marianne P. Allen/
Primary Examiner, Art Unit 1647
/R. M. D./
Examiner, Art Unit 1647
8/25/10